



qScript™ One-Step Fast qRT-PCR Kit, ROX™

Cat No. 95080-100 Size: 100 x 20-µL reactions
 95080-500 500 x 20-µL reactions

**Store at -25°C to -15°C
 protected from light**

Description

The qScript One-Step Fast qRT-PCR Kit, ROX is a convenient and highly sensitive solution for reverse transcription quantitative PCR (RT-qPCR) of RNA templates using hybridization probe detection chemistries such as TaqMan® 5'-hydrolysis probes or molecular beacons on Applied Biosystems 7000, 7300, 7700, 7900HT StepOne™, or StepOnePlus™ instruments. cDNA synthesis and PCR amplification are carried out in the same tube without opening between procedures. It is ideal for highly sensitive quantification of RNA viruses or low abundance RNA targets as well as high throughput gene-expression studies. The system has been optimized to deliver maximum RT-qPCR efficiency, sensitivity, and specificity in reduced reaction volumes and fast cycle times.

The One-Step Fast Master Mix, ROX is provided as a 4X concentrated solution to allow addition of higher amounts of RNA template and improved detection sensitivity with low concentration samples. The unique formulation maximizes the activities of both reverse transcriptase and Taq DNA polymerase while minimizing the potential for primer-dimer and other non-specific PCR artifacts. This enables unbiased co-amplification of low copy transcripts in the presence of higher copy reference genes in duplexed RT-qPCR applications.

Highly specific amplification is crucial to successful RT-qPCR as non-specific product(s) can compete for amplification of the target sequence and impair PCR efficiency. A key component of this kit is AccuStart™ Taq DNA polymerase, which contains monoclonal antibodies that bind to the polymerase and keep it inactive during reaction assembly and the 50°C reverse transcription step. A brief 30 second heat activation step at 95°C irreversibly denatures the antibodies, releasing fully active, unmodified Taq DNA polymerase. Rapid recovery of fully active, unmodified Taq DNA polymerase is critical for efficient extension kinetics. Replication of fragments up to 200 bp is complete in less than 20s at 60°C. The qScript One-Step Fast qRT-PCR Kit, ROX affords greater reagent economy and laboratory throughput on conventional or rapid ramp rate qPCR systems.

For minor groove binder (MGB) modified probes, we recommend the qScript One-Step Fast MGB qRT-PCR Kit, ROX.

Instrument Compatibility

Different real-time PCR systems employ different strategies for the normalization of fluorescent signals and correction of well-to-well optical variations. It is critical to match the appropriate qPCR reagent to your specific instrument. The qScript One-Step Fast qRT-PCR Kit, ROX contains an internal reference dye that has been optimized for use on Applied Biosystems 7000, 7300, 7700, 7900, 7900HT, StepOne, or StepOnePlus. Please consult the following table, or visit our web site at www.quantabio.com to find an optimized kit for your instrument platform(s).

Reagent	Cat Nos	Compatible Real-Time PCR Systems
qScript One-Step Fast qRT-PCR Kit, ROX	95080-100, 95080-500	Applied Biosystems 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne™, StepOnePlus™
qScript One-Step Fast qRT-PCR Kit, Low ROX	95081-100, 95081-500	Applied Biosystems 7500, 7500 Fast, ViiA™ 7 Stratagene MX4000™, MX3005P™, MX3000P™
qScript One-Step Fast qRT-PCR Kit	95079-100, 95079-500	Bio-Rad CFX96™, CFX384™, iCycler iQ®, iQ™5, MyiQ™ Opticon™, MiniOpticon™, Chromo4™ Cepheid Smart Cycler®; Qiagen/Corbett Rotor-Gene® Eppendorf Mastercycler® ep realplex Roche Applied Science LightCycler® 480

Components

Reagent	Description
qScript One-Step Fast RT	Optimized 20X formulation of recombinant MMLV reverse transcriptase for one-step Fast qRT-PCR.
One-Step Fast Master Mix, ROX (4X)	4X reaction buffer containing dNTPs, magnesium chloride, AccuStart Taq DNA polymerase, ROX Reference Dye, and stabilizers
Nuclease-free water	

Storage and Stability

Store components in a constant temperature freezer at -25°C to -15°C protected from light upon receipt. For lot specific expiry date, refer to package label, Certificate of Analysis or Product Specification Form.

Guidelines for One-Step qRT-PCR

- The design of highly specific primers and probes is a critical parameter for successful One-Step RT-qPCR. The use of computer aided primer design programs is encouraged in order to minimize the potential for internal secondary structure and complementation at 3'-ends within each primer, the primer pair, and primer/probe combinations. Regions of strong RNA secondary structure should be avoided as this can interfere with primer hybridization and/or impede procession of the reverse transcriptase. For best results, amplicon size should be between 70 and 150 bp. Optimal results may require titration of primer concentration between 400 and 900 nM. A final concentration of 450 nM each primer and 100 to 150 nM probe is effective for most applications. The efficacy and efficiency of any primer/probe set should be validated under fast cycling and/or rapid ramp rate protocols before use in RT-qPCR studies.
- Thaw all components, except qScript One-Step Fast RT, at room temperature. Mix vigorously, then centrifuge to collect contents to the bottom of the tube before using. Place all components on ice after thawing.
- To maximize specificity, reactions should be assembled on ice. AccuStart Taq DNA polymerase is inactive prior to high temperature activation; however, qScript One-Step reverse transcriptase is active at lower temperatures. First-strand synthesis can be carried out between 42°C and 52°C. Optimal results are generally obtained with a 5-minute incubation at 48 – 50°C. We recommend a minimum of 30s incubation at 95°C to inactivate the RT and activate AccuStart Taq prior to PCR cycling.
- Preparation of a reaction cocktail is recommended to reduce pipetting errors and maximize assay precision. Assemble the reaction cocktail with all required components except RNA template and dispense equal aliquots into each reaction tube. Add RNA to each reaction as the final step. Addition of sample as 5 to 10-µL volumes will improve assay precision.
- Suggested input quantities of template are: 1 pg to 1 µg total RNA; 10 fg to 100 ng poly A(+) RNA; 10 to 1x10⁸ copies viral RNA.
- After sealing each reaction, vortex gently to mix contents. Centrifuge briefly to collect components at the bottom of the reaction tube.

Reaction Assembly

Component	Volume for 20-µL rxn.	Final Concentration
One-Step Fast Master Mix, ROX (4X)	5 µL	1X
Forward primer	variable	400 – 900 nM
Reverse primer	variable	400 – 900 nM
Probe	variable	50-200 nM
Nuclease-free water	variable	
RNA template	5 to 10 µL	variable
qScript One-Step Fast RT	<u>1 µL</u>	1X
Final Volume (µL)	20 µL	

Note: For smaller, or larger, reaction volumes scale all components proportionally.

Reaction Protocol

Incubate complete reaction mix in a real-time thermal detection system as follows:

cDNA Synthesis	48 – 50°C, 5 min
Initial denaturation	95°C, 30s
PCR cycling (30 - 45 cycles)	95°C, 3s
	60°C, 20 to 30s (data collection step)



Quality Control

Kit components are free of contaminating DNase and RNase. The qScript One-Step Fast qRT-PCR Kit, ROX is functionally tested in RT-qPCR. Kinetic analysis must demonstrate linear resolution over six orders of dynamic range ($r^2 > 0.995$) and a PCR efficiency $> 90\%$

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